

CLAIMS

1. A method of modulating (as defined) the expression of a target gene (as defined) in a plant cell, tissue or organ comprising (a) providing one or more dispersed or foreign nucleic acid molecules (as defined) which include multiple copies (as defined) of a nucleotide sequence, each of which is substantially identical (as defined) to or complementary to the nucleotide sequence of the target gene or a region thereof, and (b) transfecting the plant cell, tissue or organ with the dispersed or foreign nucleic acid molecules for a time and under conditions sufficient for expression of at least two of the multiple copies.
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2. The method of claim 1 wherein either (a) at least two of the copies are in tandem and the same orientation, or (b) at least one of the copies is in the sense orientation and one is in the antisense orientation and these two copies are located relative to each other such that the two copies may form a hairpin RNA structure when transcribed.
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3. A method according to claim 2 wherein the modulating is at least partly post-transcriptional.
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4. The method according to claim 2 wherein at least one of the copies is a reverse complement of another of the copies.
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5. The method according to claim 2 wherein the copies include both direct and inverted repeats of the target gene sequence or a region thereof or complementary thereto.
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6. The method of claim 1 wherein at least two of the copies are separated by a stuffer fragment which comprises a sequence of nucleotides, or a homologue, analogue or derivative thereof.

7. A method according to claim 6 wherein the modulating is at least partly post-transcriptional.
8. The method according to claim 2, wherein the number of copies is two.
9. The method according to claim 2, wherein the number of copies is four.
- 5 10. The method according to claim 2, wherein the number of copies is ten.
11. The method according to claim 1 wherein at least one of the copies is capable of encoding an amino acid sequence encoded by the target gene.
12. The method according to claim 1 wherein the plant is a tobacco plant.
13. The method according to claim 1 wherein the target gene is a gene which is contained within the genome of the cell, tissue or organ.
- 10 14. The method according to claim 1 wherein the target gene is derived from the genome of a pathogen of the cell, tissue or organ or an organism comprising said cell, tissue or organ.
15. The method according to claim 14 wherein the pathogen is a virus.
- 15 16. The method according to claim 15 wherein the virus is a plant pathogen.
17. The method according to claim 15 wherein the virus is PVY.
18. A method of modulating (as defined) the expression of a target gene (as defined) in a plant cell, tissue or organ, said method comprising:
 - (v) selecting one or more dispersed nucleic acid molecules or foreign nucleic acid molecules (as defined) which comprise multiple copies (as defined) of a nucleotide sequence which is substantially identical
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(as defined) to the nucleotide sequence of said target gene or a region thereof or which is complementary thereto;

5 (vi) producing a synthetic gene comprising said dispersed nucleic acid molecules or foreign nucleic acid molecules operably connected to a promoter sequence operable in said cell, tissue or organ;

(vii) introducing said synthetic gene to said cell, tissue or organ; and

(viii) expressing said synthetic gene in said cell, tissue or organ for a time and under conditions sufficient for expression of at least two of the copies.

10 19. The method of claim 18 wherein either (a) at least two of the copies are in tandem and the same orientation, or (b) at least one of the copies is in the sense orientation and one is in the antisense orientation and these two copies are located relative to each other such that the two copies may form a hairpin RNA structure when transcribed.

15 20. A method according to claim 19 wherein the modulating is at least partly post-transcriptional.

21. The method of claim 20 wherein at least two of the copies are separated by a stuffer fragment which comprises a sequence of nucleotides, or a homologues, analogues or derivatives thereof.

20 22. A method of conferring resistance or immunity to a viral pathogen upon a plant cell, tissue, organ or whole organism, comprising:

(v) selecting one or more dispersed nucleic acid molecules or foreign nucleic acid molecules (as defined) which comprise multiple copies (as defined) substantially identical to a nucleotide sequence derived

from the viral pathogen or a complementary sequence thereto or a region thereof;

5 (vi) producing a synthetic gene comprising said dispersed nucleic acid molecules or foreign nucleic acid molecules operably connected to a promoter sequence operable in said cell, tissue, organ or whole organism;

10 (vii) introducing said synthetic gene to said cell, tissue, organ or whole organism; and

15 (viii) expressing said synthetic gene in said cell, tissue or organ for a time and under conditions sufficient for expression of at least two of the copies.

23. The method according to claim 22, wherein the dispersed nucleic acid molecules or foreign nucleic acid molecules comprise multiple copies of nucleotide sequence encoding a viral replicase, polymerase, coat protein or uncoating gene.

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24. The method according to claim 23 wherein the dispersed nucleic acid molecules or foreign nucleic acid molecules comprise multiple copies of nucleotide sequence encoding a viral polymerase or viral coat protein.

25. A genetic construct comprising multiple structural gene sequences (as defined), wherein each of said structural gene sequences is substantially identical (as defined) to a target gene (as defined) in a plant cell, and wherein said multiple structural gene sequences are placed operably under the control of a single promoter sequence which is operable in said cell, wherein at least one of said structural gene sequences is placed operably in the sense orientation under the control of said promoter sequence and at least one other of said structural gene sequences is placed operably in the antisense orientation under the control of said promoter sequence, and

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wherein at least one structural gene sequence that is placed in the sense orientation relative to said promoter and at least one structural gene sequence that is placed in the antisense orientation relative to said promoter are spaced from each other by a nucleic acid stuffer fragment.

5 26. The genetic construct of claim 25, wherein the transcription product of at least one structural gene sequence that is in the sense orientation relative to the promoter, said stuffer fragment and at least one structural gene sequence that is in the antisense orientation relative to the promoter may form a hairpin RNA structure when transcribed.

10 27. The genetic construct of claim 26 further comprising at least one of an origin of replication or a selectable marker gene.

28. The genetic construct according to claim 25 selected from the list comprising plasmid pSP72.PVYx2; plasmid pBC.PVYx2; plasmid pBC.PVYx3; plasmid pBC.PVYx4; plasmid pART27.PVYx2; plasmid pART27.PVYx3; plasmid pART27.PVYx4; plasmid pBC.PVY.LNYV.YVP Δ ; plasmid pBC.PVY.LNYV.YVP; plasmid pBC.PVY.LNYV.PVY; plasmid pART27.PVY.LNYV.PVY; plasmid pART27.PVY.LNYV.YVP Δ ; plasmid pART27.PVY.LNYV.YVP; plasmid pART27.35S.PVY.SCBV.YVP; plasmid pART27.35S.PVYx3.SCBV.YVPx3; plasmid pART27.PVYx3.LNYV.YVPx3; and plasmid pART27.PVYx10.

15 29. Use of the genetic construct according to claim 28 to confer immunity or resistance against PVY upon a plant cell, tissue, organ or whole plant.

20 30. Use according to claim 29, wherein the plant is tobacco.

31. A genetic construct which is capable of modulating (as defined) the expression of a target gene in a plant cell, which is transfected with said construct, wherein said construct comprises multiple copies (as defined) of a structural gene sequence (as defined), wherein each copy comprises a

nucleotide sequence which is substantially identical to said target gene or a derivative of said target gene and wherein said multiple copies are placed operably under the control of a single promoter sequence which is operable in said cell, wherein at least two of said copies are placed operably in the sense orientation under the control of said promoter sequence.

32. A genetic construct which is capable of modulating (as defined) the expression of a target gene (as defined) in a plant cell, which is transfected with said construct, wherein said construct comprises multiple structural gene sequences (as defined) wherein each of said structural gene sequences is separately placed under the control of a promoter which is operable in said cell, and wherein each of said structural gene sequences comprises a nucleotide sequence which is substantially identical to said target gene or a derivative of said target gene, wherein at least one of said structural gene sequences is placed operably in the sense orientation under the control of an individual promoter sequence.